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90. The method of claim 85, wherein said enhanced antibody, or functional fragment thereof, has an association rate constant (k_{on}) that is $6.8 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$ or higher.

91. The method of claim 90, wherein said enhanced antibody, or functional fragment thereof, has an association constant (K_a) that is $2.0 \times 10^9 \text{ M}^{-1}$ or higher.

92. The method of claim 85, wherein said enhanced antibody, or functional fragment thereof, is a grafted antibody, or functional fragment thereof.

REMARKS

Claim 1 is pending in the above-identified application. By the present communication, claim 1 has been cancelled and new claims 80-92 have been added. A marked up copy of the amendments to the specification is provided in Appendix A.

The title page and specification have been amended to change the title of the application. Support for the amendment can be found in the application, for example, on page 17, lines 3-6; page 24, lines 9-18; page 27, line 32, through page 28, line 2, and page 30, lines 17-23.

The specification has been amended to correct various typographical errors. Support for the amendments can be found throughout the specification. In particular, support for the amendment on page 14 to "Chothia" can be found, for example, on

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page 13, line 18, and page 14, line 2. Support for the amendment on page 64 can be found on page 65, lines 7-8, and Figure 2a, which shows that SEQ ID NO:11 shown on page 65 corresponds to nucleotide 139. Support for the amendment on page 87 to "Table 8" can be found, for example, on page 80, line 4, which shows that the previous table is number 7, and on page 91, line 1, which shows that the subsequent table is number 9. Support for the amendment on page 97 can be found, for example, on page 91, lines 2-3, and on page 97, line 4, which shows that the indicated values are for k_{off} . The amendment on page 16 corrects an obvious typographical error. Those skilled in the art would not only have recognized these typographical errors but also the corrections made by amendment herein.

New pages 101 through 130 are submitted herewith containing Sequences 1 through 100, formatted in accordance with the conventions set forth by PatentIn. No new matter is introduced by these new pages as they merely represent the sequences originally set forth in the prior application U.S. Serial No. 09/016,061.

Support for new claims 80-92 can be found in the specification, for example, on page 29, lines 3-15, which teaches that an antibody of the invention can be enhanced to have greater than 2 fold to 5 fold higher affinity than a reference antibody; page 28, lines 20-32, which teaches that a reference antibody can be the parent antibody from which the enhanced antibody, having increased affinity, is produced; page 95, line 30, through page 96, line 3, which teaches that an antibody with improved affinity

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can have 2 to 4 fold greater association rates and Table 10 on page 97, which provides a reference antibody having an association rate constant (k_{on}) of $6.8 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$ and association constant (K_a) of 2.5×10^9 . The specification describes dissociation constants (K_d) M^{-1} . The association constant (K_a) is merely the mathematical inverse of the dissociation constant (i.e. $K_a = 1/K_d$). Further support can be found in Table 10 which provides examples of 7 enhanced antibody fragments, including 8F9, C29, 2G4, 6H6, C37, 6D1 and 6G1, having greater than 2 fold higher affinity and greater than 2 fold higher k_{on} compared to the reference, wild type antibody fragment. Support for new claims 80-92 can also be found in the specification, for example, on page 12, lines 9-28; page 17, lines 3-6; page 30, line 1, through page 31, line 6, and page 89, line 17, through page 90, line 13. Accordingly, these amendments do not introduce any new matter and entry thereof is respectfully requested.

Regarding Priority

As indicated in the attached transmittal, Applicants herewith have amended the specification to delete the claim to priority under 35 U.S.C. § 120, which indicated that the above-identified application is a continuation-in-part of U.S. Application 08/791,391, filed January 30, 1997. Accordingly, Applicants hereby claim priority to the actual filing date for application Serial No. 9/106,061, January 30, 1998.

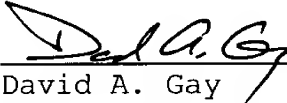
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CONCLUSION

The amendments and new claims added herein are supported in the specification and do not add new matter. Accordingly, Applicants respectfully request entry of the amendments and new claims. If there are any questions, the Examiner is invited to call Cathryn Campbell or the undersigned agent at (858) 535-9001.

Respectfully submitted,

July 6, 2001
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Appendix A

Version with markings to show changes made to the title starting on line 5 of the title page:

COMPOSITIONS AND METHODS FOR PRODUCING ENHANCED ANTIBODIES [ANTI- $\alpha_v\beta_3$ RECOMBINANT HUMAN ANTIBODIES, NUCLEIC ACIDS ENCODING SAME AND METHODS OF USE]

Version with markings to show changes made to the title starting on line 1 of page 1:

COMPOSITIONS AND METHODS FOR PRODUCING ENHANCED ANTIBODIES [ANTI- $\alpha_v\beta_3$ RECOMBINANT HUMAN ANTIBODIES, NUCLEIC ACIDS ENCODING SAME AND METHODS OF USE]

Version with markings to show changes made to footnote 2 starting on page 14, line 11 and ending on page 14, line 12:

² Residue numbering follows the nomenclature of Chothia [Clothia] et al., *supra*

Version with markings to show changes made to the paragraph starting on page 16, line 10:

As used herein, the term "functional fragment" when used in reference to Vitaxin, to a LM609 grafted antibody or to heavy or light chain polypeptides thereof is intended to refer to

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a portion of Vitaxin or a LM609 grafted antibody including heavy or light chain polypeptides which still retains some or all of [or] the $\alpha_v\beta_3$ binding activity, $\alpha_v\beta_3$ binding specificity and/or integrin $\alpha_v\beta_3$ -inhibitory activity. Such functional fragments can include, for example, antibody functional fragments such as Fab, F(ab)₂, Fv, single chain Fv (scFv). Other functional fragments can include, for example, heavy or light chain polypeptides, variable region polypeptides or CDR polypeptides or portions thereof so long as such functional fragments retain binding activity, specificity or inhibitory activity. The term is also intended to include polypeptides encompassing, for example, modified forms of naturally occurring amino acids such as D-stereoisomers, non-naturally occurring amino acids, amino acid analogues and mimetics so long as such polypeptides retain functional activity as defined above.

Version with markings to show changes made to the paragraph starting on page 64, line 1:

Grafted LM609 heavy and light chain V regions were constructed by mixing 5 overlapping oligonucleotides at equimolar concentrations, in the presence of annealing PCR primers. The heavy chain oligonucleotides map to the following nucleotide positions: V_H oligonucleotide 1 (V_H oligo1), nucleotides (nt) 1-84; (SEQ ID NO:9); V_H oligo2, nt 70-153, (SEQ ID NO:10); V_H oligo3, nt 139[138]-225 (SEQ ID NO:11); V_H oligo4, nt 211-291 (SEQ ID NO:12); V_H oligo5, nt 277-351 (SEQ ID NO:13). Similarly, the Vitaxin light chain oligonucleotides map to the following

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nucleotide positions: V_L oligonucleotide 1 (V_L oligo1),
nucleotides (nt) 1-87; (SEQ ID NO:14); V_L oligo2, nt 73-144, (SEQ
ID NO:15); V_L oligo3, nt 130-213 (SEQ ID NO:16); V_L oligo4, nt
199-279 (SEQ ID NO:17); V_L oligo5, nt 265-321 (SEQ ID NO:18).
The nucleotide sequences of oligonucleotides used to construct
grafted LM609 heavy and light chain variable regions are shown in
Table 6. Codon positions 49 and 87 in V_L oligo3, and V_L oligo4
represent the randomized codons. The annealing primers contained
at least 18 nucleotide residues complementary to vector sequences
for efficient annealing of the amplified V region product to the
single-stranded vector. The annealed mixture was fully converted
to a double-stranded molecule with T4 DNA polymerase plus dNTPs
and ligated with T4 ligase.

Version with markings to show changes made to the
paragraph starting on page 83, line 27:

Oligonucleotides encoding a single mutation were
synthesized by introducing NN(G/T) at each CDR position as
described previously (Glaser et al., *supra*). The antibody
libraries were constructed in M131XL604 vector by hybridization
mutagenesis as described previously, with some modifications
(Rosok et al., J. Biol. Chem. 271:22611-22618 (1996); Huse et
al., J. Immunol. 149:3914-3920 (1992); Kunkel, Proc. Natl. Acad.
Sci. USA 82:488-492 (1985); Kunkel et al., Methods Enzymol.
154:367-382 (1987)). Briefly, the oligonucleotides were annealed
at a 20:1 molar ratio to uridynylated LM609 grafted antibody
template (from which the corresponding CDR had been deleted) by

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denaturing at 85°C for 5 min, ramping to 55°C for 1 h, holding at 55°C for 5 min, then chilling on ice. The reaction was extended by polymerization and electroporated into DH10B and titered onto a lawn of XL-1 Blue. The libraries consisted of pools of variants, each clone containing a single amino acid alteration in one of the CDR positions. Utilizing codon-based mutagenesis, every position in all of the CDRs was mutated, one at a time, resulting in the subsequent expression of all twenty amino acids at each CDR residue (Glaser et al., *supra*). The CDR libraries ranged in size from 288 (L3) to 416 (L1) unique members and contained a total of 2336 variants.

Version with markings to show changes made to the title to the table starting on page 87, line 8:

Table 8 [10]: Capture Lift Screening of LM609 grafted antibody CDR Libraries.

Version with markings to show changes made to footnote 1 starting on page 87, line 18, and ending at line 20:

¹Number of unique clones based on DNA sequence. Thirty-two codons [condons] are used to express all twenty amino acids at each position.

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Version with markings to show changes made to the Table on page 97:

Table 10: Identification of Optimal Combinatorial Mutations

library*	clone	L1 32	L3 92	L3 96	sequencet H2 60 H3 97 H3 101 H3 102				k_{on} ($\times 10^4$) ($M^{-1}s^{-1}$)	k_{off} [k_{on}] ($\times 10^{-3}$) (s^{-1})	Kd (nM)
wild type		H	G	H	L	Y	A	Y	18.0	4.97	27.6
F32	17	F						S	25.1	0.138	0.5
	7	F			P	H		S	20.4	0.236	1.2
	56	F			P			S	26.6	0.135	0.5
	C59	F			P			D	26.5	0.137	0.5
	C176	F			P			T	22.5	0.192	0.9
	V357D	F						D	27.9	0.140	0.5
N92	C119		N		P			S	21.5	0.316	1.5
L96	8F9			L	P	H		S	47.5	0.280	0.6
	C29			L	P	H	Y	S	67.5	0.343	0.5
	2G4			L				S	60.3	0.229	0.4
	6H6			L		H		S	50.4	0.187	0.4
	C37			L			Y	E	44.8	0.147	0.3
	6D1			L	P		Y	S	41.0	0.158	0.4
	6G1			L	P			S	38.9	0.280	0.7